UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

APR 20 1992

MEMORANDUM

SUBJECT TRIISOPROPANOLAMINE Salt of 2,4-Dichlorophenoxyacetic acid: Generic Data Submission: Toxicology Guideline-82-1(a).

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 4/8/92
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TO: Walter Waldrop / Judy Coombs
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THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 4/9/92
Section II, Toxicology Branch II
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and
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TASK IDENTIFICATIONS: Case No: 818722 Submission No: 8406175

HED Project No. 2-0320 Caswell No. 315-AE

Registrant: DowElanco, 9002 Purdue Rd, Indianapolis, IN

ACTION REQUESTED: Review of a subchronic toxicity study in rats with 2,4-D TIPA.

RESPONSE: A Data Evaluation Report [DER] is attached and the findings are summarized below.

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SUMMARY: Groups of 10 male and 10 female Fischer-344 rats were fed diets containing the Triisopropanolamine salt of 2,4-Dichlorophenoxyacetic acid at 0, 2, 28, 187, or 560 mg/kg/day for 13 weeks.

No treatment-related effects were seen at 2 or 28 mg/kg/day. Treatment-related effects observed at 187 mg/kg/day included: decreases in mean body weight gain [females]; minor alterations in hematology [decreases in red blood cell and platelet counts in females]; clinical chemistry [increases in ALT activity, AST activity, albumin, triglycerides and decreased T_4 in females]; urinalysis [decrease in specific gravity in both sexes]; and slight histopathologic changes in the liver and kidneys of males, and in the kidneys and adrenal glands of females.

Primary treatment-related effects observed at 560 mg/kg/day included: decreases in mean body weight, body weight gain and food consumption [both sexes]; alterations in hematology [decreases in red blood cell, white blood cell and platelet counts and decreases in hemoglobin and hematocrit concentrations in both sexes]; clinical chemistry [decreases in total protein, glucose, globulin, calcium, and T_4, and increases in AST activity and cholesterol in males, and decreases in total protein, globulin, glucose, calcium and T_4, and increases in BUN, AP, ALT, AST, triglycerides, albumin, and T_3 in females]; urinalysis [decrease in specific gravity in both sexes]; changes in organ weights [increase in relative kidney, liver and thyroid weights in both sexes]; gross pathology; and severe histopathological changes in the eyes, kidneys, liver and thyroids of male and female rats. Effects secondary to decreased weight gain, the debilitated condition of the rats, and/or toxicity in other organs occurred in the adrenals, bone marrow, fat [mesenteric], lungs, spleen, thymus, and testes.

Under the conditions of this study, a NOEL of 28 mg/kg/day and a LOEL of 187 mg/kg/day is established for the 90-day oral toxicity of the Triisopropanolamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight gain, alterations in hematology, clinical chemistry and urinalysis, increase in relative kidney weights, and mild histopathological lesions in the kidneys and liver of males, and in the kidneys and adrenal glands of females.

VI. CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 4/8/92
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SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 4/9/92
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DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

TOX.CHEM. No.: 315-AE **MRID No.:** 420214-02

HED Project No. 2-0320 **Registrant:** DowElanco.

TEST MATERIAL: Triisopropanolamine Salt of 2,4-D (2,4-D TIPA)

TESTING LABORATORY: Toxicology Research Lab, Dow Chem. Co.

STUDY IDENTIFICATION: K-008866-006

TITLE OF REPORT: 2,4-D Triisopropanolamine Salt [2,4-D TIPA]: A 13-Week Dietary Toxicity Study in Fischer 344 rats.

REPORT AUTHORS: B. L. Yano, P.F. Cosse, L. Atkin and R.A. Corley

STUDY COMPLETION DATE: September 4, 1991.

SUMMARY: In groups of 10 male and 10 female Fischer-344 rats fed diets containing the Triisopropanolamine salt of 2,4-Dichlorophenoxy-acetic acid at 0, 2, 28, 187, or 560 mg/kg/day for 13 weeks, no treatment-related effects were seen at 2 or 28 mg/kg/day. Treatment-related effects observed at 187 mg/kg/day were: decreases in mean body weight gain; alterations in hematology, clinical chemistry and urinalysis; changes in organ weights; and mild histopathologic changes in the liver, kidneys and adrenal glands.

Primary treatment-related effects observed at 560 mg/kg/day included: decreases in mean body weight and body weight gain and food consumption; alterations in hematology, clinical chemistry and urinalysis; changes in organ weights, gross pathology; and histopathological changes in the eyes, kidneys, liver and thyroids of male and female rats. Effects secondary to decreased weight gain, the debilitated condition of the rats, and/or toxicity in other organs occurred in the adrenals, bone marrow, fat [mesenteric], lungs, spleen, thymus, and testes.

Under the conditions of this study, a NOEL of 28 mg/kg/day and a LOEL of 187 mg/kg/day is established for the 90-day oral toxicity of the Triisopropanolamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

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I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of the Triisopropanolamine salt of 2,4-Dichlorophenoxyacetic acid [2,4-D TIPA] following dietary administration to rats.

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: Triisopropanolamine salt of 2,4-D.
Purity: 72.2% TIPA and 38.7% 2,4-D acid.
Lot No.: AGR 276428
Description: Aqueous amber liquid.
Vehicle: Acetone

2. Test Animals

Species: Rats
Strain: Fischer-344
Sex: Males and females
Weight at Initiation: 70 - 83 g (M); 60 - 75 g (F)
Identification: Ear tags.
Acclimation: Minimum of seven days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature and humidity controlled rooms with a photocycle regulated for rats.

3. Study Design

Group No.	Treatment	No. of Animals		Dose level [mg/kg/day]	Acid Equivalent [mg/kg/day]
		Males	Females		
1	Control	10	10	0	0
2	Low	10	10	2	1
3	Mid-1	10	10	28	15
4	Mid-2	10	10	187	100
5	High	10	10	560	300

4. Test Article Formulation and Analyses

a. Formulation: Test material was dissolved in a relatively small amount of acetone to facilitate its uniform dispersion in the diet. The test diets were prepared weekly by serial dilution of the high-dose to attain the lower doses. Initial concentrations of test material in the diet were calculated from the pretest body weights and feed consumption data. Thereafter, the most recent body weight and food consumption data were used to adjust the concentration of the test material in the diet to maintain the dose levels on the targeted mg/kg/day basis. Reference samples [one/dose/sex/mix plus premix] were retained and stored at ambient temperature.

b. Analysis: Difficulties were encountered in analyzing the diets for 2,4-D TIPA due to the complete disassociation of the test material to the conjugate base [2,4-D] and ammonium ion [TIPA] moieties in aqueous solution. Therefore, verification of the stability of 2,4-D TIPA in test diets were conducted in two separate analyses based on the stability of the 2,4-D acid and TIPA moieties for at least 21 days. Homogeneity and concentration analysis based on analysis of 2,4-D acid were performed at the start of the study and monthly thereafter.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed once daily. Body weights and food consumption were measured prior to initiation and once weekly. A complete physical examination was performed weekly. Ophthalmologic examinations were conducted prior to initiation [Day - 5] and at termination [Day-90]. A functional observation battery [FOB] was conducted on all surviving rats on Day 90. Blood and urine were collected from all animals at termination for hematology, clinical chemistry and urinalysis. The checked (x) parameters were determined.

Hematology

x Hematocrit (HCT)[a]	x Leukocyte count (WBC)[a]
x Hemoglobin (HGB)[a]	x Platelet count[a]
x Erythrocyte count (RBC)[a]	x Leukocyte differential[a]
x Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume (MCV)	Blood clotting measurements
Corrected leukocyte count (COR WBC)	x Cell morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a]	x Albumin[a]
x Chloride[a]	x Blood creatinine[a]
Magnesium[a]	x Blood urea nitrogen[a]
x Phosphorus[a]	x Cholesterol[a]
x Potassium[a]	x Globulins
x Sodium	x Glucose[a]
	x Total bilirubin[a]
Enzymes:	x Total serum protein[a]
	x Triglycerides
x Alkaline phosphatase	Serum protein electrophoresis
x Alanine aminotransferase (SGPT)[a]	x Triiodothyronine [T_3]
x Aspartate aminotransferase (SGOT)[a]	x Thyroxine [T_4]
Cholinesterase[b]	x A/G Ratio
x Creatinine phosphatase[a]	
Lactic acid dehydrogenase	
Gama glutamyl transferase	

Urinalysis

x Appearance[c]	x Color	x Bilirubin[c]
x Specific gravity[c]		x Occult blood[c]
x pH	Volume	x Urobilinogen
x Protein	Nitrites	x Glucose[c]
x Ketones[c]		x Microscopic examination of sediment[c]

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.
[c] Required for chronic studies.

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7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys
Liver	Ovaries	Thyroid	Testes

8. Histopathology

The checked (X) tissues from all animals in the control and high-dose groups were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Duodenum[a]	Pharynx[e]
x Jejunum[a]	Larynx[e]
x Cecum[a]	Nose[e]
x Colon[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	x Aorta (thoracic)[a]
Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
x Peripheral nerve[ac]	Urinogenital System
x Spinal cord (3 levels)[ac]	
x Eyes (optical nerve)[ac]	x Kidneys[ac]
	x Urinary bladder[a]
Glandular System	x Testes[ac]
	x Epididymides
x Adrenals[a]	x Uterus[a]
Lacrimal glands[c]	x Ovaries[ac]
x Parathyroids[ad]	Others
x Thyroids[ad]	
	x All gross lesions and masses
	x Skeletal muscle[a]

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

In addition, the liver, kidneys, lung, thyroid, adrenal, thymus, mesenteric lymph node, eyes, bone marrow with bone, cecum, colon, mesenteric tissue, spleen, testes, ovaries and all gross lesions from the lower dose groups also underwent histopathological examination.

9. Statistical Analyses

Only means and standard deviations were reported for food consumption, WBC differential counts, RBC indices and reticulocyte counts. Bartlett's test for equality of variances was employed for body weights, organ weights, clinical chemistry data, appropriate hematology data and urinary specific gravity. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance [ANOVA], followed respectively by Dunnnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of The Toxicology Research Laboratory, and the Study Protocol. A quality assurance statement was signed and dated August, 28, 1991.

XII. RESULTS

1. Analysis of Diet Mix

Concentrations of test diets, based on analysis of 2,4-D indicated that the diets were accurately prepared throughout the study. The mean assay values were 116%, 115%, 98% and 104% respectively, of the nominal concentrations for males and 116%, 119 %, 104% and 102% respectively, of the nominal concentrations for females at 2, 28, 187 and 560 mg/kg/day diets.

Homogeneity of the test diets, based on analysis of 2,4-D indicated that diets formulated to deliver 112 or 187 mg/kg/day were homogenous. The mean values [% of target] were 89% and 47% respectively for the 112 and 187 mg/kg/day diets.

Stability analyses for the acid moiety indicated that the test material was stable with the recovery of 90.38 ± 1.30% and 110.88 ± 2.28% acid and amine moieties, respectively after 21 days in separate experiments using ^{14}C-labelled 2,4-D acid and ^{14}C-labeled TIPA.

2. Survival

All rats survived the study.

3. Clinical Observations

Treatment-related clinical signs of toxicity were limited to unilateral or bilateral opaque eyes noted in 8 males and 10 females at 560 mg/kg/day.

4. Functional Observational Battery:

Except for opaque eyes [lens] identical to those noted on in males and females at 560 mg/kg/day during ophthalmic examination [see below], no other treatment-related effects were seen in either sex of rats at any dose levels.

5. Ophthalmology Examination

Bilateral lens opacities that were considered to be cataracts were seen in 8 males and 10 females at 560 mg/kg/day. The differences in the incidences of corneal opacities between the treated and control group showed no dose response.

6. **Body Weights and Body Weight Changes**

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Statistical [$p<0.05$] differences were seen in:

- o Males at 560 mg/kg/day from Weeks 2 - 13,
- o Females at 560 mg/kg/day from Weeks 1 - 13;
- o Females at 187 mg/kg/day from Weeks 3 - 6.

Mean body weights of males and females at 2 and 28, and in males at 187 mg/kg/day were comparable to that of the control rats.

Table 1. Mean Body Weights and S.D (G) for Male Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13*
0	103.0 ± 4.8	230.2 ± 15.6	280.0± 11.9	291.7± 10.6
2.0	102.8 ± 5.5	231.6 ± 13.4	279.9± 14.5	295.6±15.8
28.0	103.0± 5.5	227.2 ± 15.9	276.1± 16.7	290.9±17.9
187.0	103.2 ± 6.0	227.5 ± 9.6	274.7± 11.8	288.5±15.3
560.0	98.7 ± 5.0	172.2* ±13.5	191.4*±21.1	191.4* ±25.2

* Significantly different from control value at $p \leq 0.05$.

Table 2. Mean Body Weights and S.D (G) for Female Rats.

Dose Level [mg/kg/day]	Week: 0	Week: 6	Week: 10	Week: 13
0	87.9 ± 2.2	145.7 ± 6.5	166.2 ± 8.4	173.9 ± 9.2
2.0	89.6 ± 4.6	148.4 ± 6.5	172.0 ± 7.3	180.8 ± 6.6
28.0	87.3 ± 4.0	149.2 ± 7.3	171.1 ± 6.4	179.4 ±6.6
187.0	85.7 ± 3.1	137.0* ± 5.6	162.1 ± 7.8	169.6 ± 8.3
560.0	80.3 ± 3.7	115.6* ± 9.9	123.9* ±14.4	120.7* ± 16.0

* Significantly different from control value at $p \leq 0.01$

Mean body weight gain of male and female rats at 2, 28, and 187 mg/kg/day were comparable to those of the respective controls. Mean body weight gain was lower for both sexes of rats at 560 mg/kg/day as shown in Tables 3 and 4.

Table 3. Mean Body Weight Change (G) in Male Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	92 ± 11	149 ± 9	189 ± 8
2.0	93 ± 13	150 ± 11	193 ± 13
28.0	87 ± 14	148 ± 11	188 ± 14
187	89 ± 8	146 ± 12	185 ± 15
560	55 ± 6	85 ± 15	93 ± 23

Table 4. Mean Body Weight Change (G) in Female Rats.

Dose Level [mg/kg/day]	Weeks: 0 - 4	Weeks: 0 - 8	Weeks: 0 - 13
0	44 ± 4	66 ± 7	86 ± 9
2	45 ± 3	69 ± 3	91 ± 4
28	47 ± 4	72 ± 4	92 ± 5
187	38 ± 3	64 ± 9	83 ± 6
560	28 ± 5	38 ± 11	40 ± 16

7. Food Consumption

No differences were seen in feed consumption of males and females at 2,28, or 187 mg/kg/day. Male and female rats at 560 mg/kg/day exhbited a 30% reduction in mean absolute [g//rat/day] food consumption values when compared to controls. The decrease in food consumption was related to the reduced body weights observed in these rats. The decrease in feed consumption may have resulted from unpalatability of the feed and/or due to slight anorexia secondary to toxicity.

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8. **Hematology and Clinical Chemistry**

Treatment-related, statistically significant [p <0.05] changes observed in hematology included decreases in red blood cell and platelet counts in females at 187 mg/kg/day, and decreases in red blood cell, white blood cell and platelet counts and decreases hemoglobin and hematocrit concentrations in male and females at 560 mg/kg/day. Alterations noted in the other hematological parameters were not considered to be treatment related since they were within normal limits and/or showed no dose response relationship.

Changes in observed in clinical chemistry parameters are summarized below:

Dose Level [mg/kg/day] Sex	Parameter Affected	Effect
187 / ♂	AST	Increased
187 / ♀	ALT	Increased
	AST	Increased
	Albumin	Increased
	Triglycerides	Increased
	T_4	Decreased
560 / ♂	AST	Increased
	Tot.Prot.	Decreased
	Globulin	Decreased
	Glucose	Decreased
	Cholesterol	Increased
	Calcium	Decreased
	T_4	Decreased
560 / ♀	BUN	Increased
	ALT	Increased
	AST	Increased
	AP	Increased
	Tot.Prot.	Decreased
	Albumin	Increased
	Globulin	Decreased
	Glucose	Decreased
	Triglycerides	Increased
	Calcium	Decreased
	T_4	Decreased
	T_3	Increased

9. **Urinalysis**

Except for the statistically significant [p <0.05], dose-related decreases in urine specific gravity in males and females at 187 and 560 mg/kg/day, urinalysis revealed no other treatment related changes.

10. **Organ Weights**

Statistically significant [p < 0.05] differences observed in the absolute and relative organ weight are summarized below:

Absolute Organ Weights			
Organ	Dose Level [mg/kg/day]	Sex	Effect
Adrenal	560	M & F	Decreased
Brain	560	M & F	Decreased
Heart	560	M & F	Decreased
Kidneys	187 560	M M & F	Increased Decreased
Liver	560	M & F	Increased
Thyroid	560	M & F	Increased
Testes	560	M	Decreased
Ovaries	560	F	Decreased

Relative Organ Weights			
Organ	Dose Level [mg/kg/day]	Sex	Effect
Adrenal	560	F	Decreased
Brain	560	M & F	Increased
Heart	560	M & F	Increased
Kidneys	187 & 560	M & F	Increased
Liver	560	M & F	Increased
Thyroid	560	M & F	Increased
Testes	560	M	Decreased
Ovaries	560	F	Decreased

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11. Gross Pathology

No treatment-related gross pathological changes were seen in either sexes at 2, 28, or 187 mg/kg/day. At 560 mg/kg/day, gross pathological changes included: lenticular opacity of the eyes [9 ♂ and 10 ♀]; accentuated lobular pattern of the liver [10 ♂ and 10 ♀]; increased liver size [2 ♂ and 7 ♀]; pale foci in the lungs [10 ♂ and 10 ♀]; dark foci in the glandular mucosa of the stomach [2 ♂ and 3 ♀]; hemolyzed blood in the lumen of the stomach [2 ♂ and 2 ♀]; increased cecal size [4 ♂ and 6 ♀]; decreased amount of general fat [10 ♂ 10 ♀]; increased thyroid gland size [3 ♂]; and decreased testicular size [10 ♂]. Other gross necropsy findings were considered incidental in nature.

12. Histopathology

No treatment-related histopathological changes were seen in male or female rats at 2 or 28 mg/kg/day. Treatment-related histopathological changes observed at 187 and 560 mg/kg/day are tabulated in Tables 5 and 6 and are summarized below:

Dose Level [mg/kg/day]	Sex	Organs with Lesions
187	M	Kidneys and Liver
	F	Adrenal, Fat [mesenteric], and Kidneys
560	M & F	Adrenals, Bone marrow, Eyes, Fat [mesenteric], Kidneys, Liver, Lungs, Lymph node [♀], Spleen, Thymus, Thyroid, and Testes [♂]

Adrenal glands: Cells of the adrenal zona gomerulosa were very slightly to slightly increased in size [hypertrophy] with the cytoplasm being more vacuolated than the controls. No microscopic lesions which corresponded with the decreased absolute and relative weights were seen.

Bone marrow: The cellularity of erythroid, myeloid and megakaryocyte cell lines in the bone marrow was slightly decreased and fat spaces were more readily observed.

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Eyes: Cataracts were mostly bilateral and diffused. Retinal degeneration was almost exclusively bilateral, diffuse, and varied from slight to severe in degree and was characterized by complete or partial loss of the rod and cone layer and the outer nuclear layer.

Kidneys: Degenerative kidney effects were very slight to slight and were characterized by a loss of the brush border of the straight descending portion of the proximal tubules, increased vacuolation of epithelial cell cytoplasm and were occasionally accompanied by focal tubular basement membrane thickening, fibrosis and individual cell atrophy.

Liver: Lesions were characterized by an increase in the size and altered tinctorial properties [increased cytoplasmic eosinophilia] of hepatocytes in the centrilobular and midzonal regions of the hepatic lobule. This effect was not accompanied by hepatocellular degeneration or necrosis.

Lungs: Very slight to slight increases in numbers of subpleural alveolar macrophages were seen.

Spleen: Generalized lymphoid atrophy was seen along with decreases in the populations of T- and B-lymphocytes. Splenic red pulp was also reduced in amount.

Thymus: Generalized lymphoid atrophy.

Thyroid: Epithelial cells lining thyroid follicles were very slightly to slightly increased in size [hypertrophy-column versus cuboidal shape] with a vacuolated cytoplasm. These alterations were most likely the result of a compensatory response due to decreased serum T_4 levels.

Testes: Testicular degeneration was moderate to severe and characterized by an absence of spermatozoa, variable decreases in the number of spermatids, spermatocytes and spermatogonia and presence of occasional multinucleated spermatids.

Other microscopical changes were considered incidental and unrelated to treatment.

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Table 5. Histopathological Findings in Male Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	2	28	187	560
Adrenals					
-Hypertrophy, Zona Glomerulosa	0	0	0	0	9
Bone Marrow					
-Hematopoiesis, decreased	0	0	0	0	9
Eye					
- Retinal degeneration	0	0	0	0	2
- Cataract,lens, bilateral	0	0	0	0	7
- Cataract,lens, unilateral	0	0	0	0	1
Kidney					
-Degeneration, Proximal Tubules	0	0	0	1	10
Liver, Centrilobular and mid zonal					
-Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
-Altered Tinctorial Properties with increased eosinophilla.	0	0	0	2	0
-Necrosis, very slight	0	0	0	1	0
Lung					
-Alveolar Histosis,	0	0	0	0	10
Mesenteric Tissue					
-Adipose tissue, Atrophy	0	0	0	0	10
Spleen					
-Atrophy	0	0	0	0	3
Testes					
-Degeneration, Tubules, Bilateral	0	0	0	0	10
Thymus					
-Atrophy	0	0	0	0	4
Thyroid					
-Hypertrophy, Epithelial cells	0	0	0	0	10

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Table 6. Histopathological Findings in Female Rats.

Organ / Lesion	Dose Level [mg/kg/day]				
No.of animals= 10 / dose levels	0	2	28	187	560
Adrenals					
-Hypertrophy, Zona Glomerulosa	0	0	0	7	10
Bone Marrow					
-Hematopoiesis, decreased	0	0	0	0	9
Eye					
-Retinal degeneration	0	0	0	1	7
-Cataract, bilateral	0	0	0	0	9
-Cataract, unilateral	0	0	0	0	1
Kidney					
-Degeneration, Proximal Tubules	0	0	0	8	10
Liver, Centrilobular and midzonal					
-Increased size of hepatocytes with Altered Tinctorial Properties	0	0	0	0	10
Lung					
-Alveolar Histosis	0	0	0	0	10
Lymph Node Mesenteric					
-Atrophy	0	0	0	0	10
Mesenteric Tissue					
-Adipose tissue, Atrophy	1	1	0	9	10
Spleen					
-Atrophy	0	0	0	0	8
Thymus					
-Atrophy	0	0	0	0	9
Thyroid					
-Hypertrophy, Epithelial cells	0	0	0	0	10

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IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing the Triisopropanolamine salt of 2,4-D at concentrations of 0, 2, 28, 187, or 560 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 21 days. The concentrations of test material in the diet approximated the targeted concentrations with the mean concentrations of target levels being between 98 to 116%.

No mortality occurred during the study. No treatment-related effects were seen at 2 or 28 mg/kg/day on mean body weight, mean body weight gain, mean food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross pathology or histopathology.

Treatment-related effects observed at 187 were: slight but statistically significant decreases in mean body weight [females]; minor alterations in hematology [decreases in RBC and platelet counts in females] and clinical chemistry parameters [increases in ALT activity, AST activity, albumin and triglycerides, and decreased T_4 levels in females]; decrease in urine specific gravity [both sexes]; changes in organ weights [increase in relative kidney weights in both sexes] and mild histopathologic changes in the liver and kidneys of males and in the kidneys and adrenal glands of females.

Primary treatment-related effects observed at 560 mg/kg/day included: decreases in mean body weight and body weight gain; a 30% reduction in food consumption; alterations in hematology, clinical chemistry, and urinalysis; changes in organ weights, gross pathology; and histopathological changes in several organs of both sexes of rats.

Treatment-related alterations in hematology parameters were limited to decreases in RBC, WBC, HCT and platelet counts and decreases in hemoglobin and hematocrit concentrations in both sexes which correlated with the decreased bone marrow cellularity observed in animals at this dose.

Treatment-related alterations in clinical chemistry parameters were decreases in Total protein, Glucose, Globulin, calcium, and T_4, and increases in AST activity and Cholesterol in males, and decreases in Total protein, Globulin, Glucose, Calcium and T_4, and increases in BUN, AP, ALT, AST, Triglycerides, Albumin, and T_3 in females. These changes correlated with histopathological lesions in the kidneys, liver, and thyroids. The remaining differences in clinical chemistry values were considered to be non treatment related and were attributed to the influence of body weight loss and the debilitated condition of the rats.

Abnormalities in urine were limited decreased specific gravity in both sexes.

Differences seen in the relative weights of kidneys, liver and thyroid were considered to be treatment related due to collaborative clinical chemistry alterations and/or histopathological changes in these tissues. Differences seen in other organ weights were attributed to effects secondary to decreased body weights and the debilitated condition of the rat at the high dose.

Although, histopathology revealed alterations in several organs such as the adrenals, bone marrow, eyes, kidneys, liver, lung, mesenteric fat, spleen, thyroid, thymus, and testes, only the changes seen in the eyes, liver, kidneys, and thyroid glands were considered to be clearly treatment-related. Alterations seen in the other organs were considered to be secondary changes possibly associated with decreased body weight, poor nutrition due to unpalatability of the food, the debilitated condition of the rats, and general toxicity involving multiple organs.

The bilateral cataracts and retinal degeneration observed in both sexes of rats at the high dose in this study were also seen in rats fed diets containing the 2,4-D acid, salts [dimethylamine, diethanolamine, and isopropylamine], and esters [butoxyethyl and 2-ethylhexyl].

The increase in size and altered tinctorial properties of hepatocytes seen in the centrilobular and midzonal regions of the hepatic nodule were not accompanied by hepatocellular degeneration and necrosis and therefore may be attributed to the induction of hepatic peroxisomes by 2,4-D TIPA. Similar liver lesions were also seen in rats fed 2,4-D IPA.

The elevated BUN values and the increase in relative kidney weights may be related to the renal alterations in the descending proximal tubules. The hypertrophy and increased vacuolation of the adrenal gland zona glomerulosa was interpreted to be a secondary change possibly associated with the renal alterations since there were no specific microscopic alterations which corresponded with the decrease in absolute and relative organ weights.

Hypertrophy of the follicular epithelial cells of the thyroid glands may be associated with a decrease in serum T_4 and increase in thyroid weights since there was no evidence of any degenerative changes involving the thyroid glands and the colloid appeared normal.

The decreased bone marrow cellularity associated with decreases in RBC, WBC and platelet counts and can be attributed to the interaction of the animals debilitated condition and possible secondary nutritional anemia. Similarly, the generalized lymphoid atrophy of the spleen and thymus, and the testicular degeneration may be attributed to poor nutrition, debilitated conditions, and general toxicity involving multiple organ systems.

The effects seen in this study were parallel to those seen in rats fed diets of 2,4-D acid at similar dose levels [1, 15, 100, or 300 mg/kg/day]. Therefore, it is suggested that the toxicity observed with 2,4-D TIPA is probably the result of the acid moiety and not the triisopropanolamine salt. Similarly, a subchronic toxicity study evaluating the effects of 2,4-D isopropylamine salt at comparable dose levels also demonstrated that the toxicity observed with 2,4-IPA was probably due to the acid moiety.

V. CONCLUSION

Under the conditions of this study, a NOEL of 28 mg/kg/day and a LOEL of 187 mg/kg/day is established for the 90-day oral toxicity of the Triisopropylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rats. The LOEL is based on decreases in mean body weight gain, alterations in hematology, clinical chemistry and urinalysis, increase in relative kidney weights and histopathological alterations in the kidneys and liver of males, and in the kidneys and adrenal glands of female rats.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.